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Abstract

Background: Acrolein is a highly reactive α, β unsaturated aldehyde and respiratory irritant. Acrolein is formed during combustion (e.g. burning tobacco or biomass), during high-temperature cooking of foods, and *in vivo* as a product of oxidative stress and polyamine metabolism. No biomonitoring reference data has been reported to characterize acrolein exposure of the U.S. population.

Objectives: Our goals were to: a) evaluate two acrolein metabolites in urine - *N*-Acetyl-S-(3-hydroxypropyl)-L-cysteine (3HPMA) and *N*-acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA) - as biomarkers of exposure to acrolein for the U.S. population by age, sex, race, and smoking status; and b) assess tobacco smoke as a predictor of acrolein exposure.

Methods: We analyzed urine from National Health and Nutrition Examination Survey (NHANES 2005-2006) participants ≥ 12 years-old (n = 2,866) for 3HPMA and CEMA using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC/ESI-MSMS). Sample-weighted linear regression models stratified for non-tobacco users vs. tobacco smokers (as defined by serum cotinine and self-report) characterized the association of urinary 3HPMA and CEMA with tobacco smoke exposure, adjusting for urinary creatinine, sex, age, and race/ethnicity.

Results: 3HPMA and CEMA levels were higher among tobacco smokers (cigarettes, cigars and pipe users) compared with non-tobacco users. The median 3HPMA levels for tobacco smokers and non-tobacco were 1089 and 219 μg/g creatinine respectively. Similarly, median CEMA levels were 203 μg/g creatinine for tobacco smokers and 78.8 μg/g creatinine for non-tobacco users. Regression analysis showed that serum cotinine was a significant positive predictor ($p < 0.0001$) of both 3HPMA and CEMA among tobacco smokers.

Conclusions: Tobacco smoke was a significant predictor of acrolein exposure in the U.S. population.

Introduction

Acrolein is a chemical contaminant ubiquitous in the environment. It is formed from carbohydrates, vegetable oils, animal fats, and amino acids during heating of foods, and by combustion of petroleum fuels and biodiesel (Stevens and Maier 2008). Additionally, 1,3 butadiene can be photo-chemically degraded into acrolein in the environment (Doyle et al. 2004). Although there are a number of sources of exposure, smoking of tobacco products is typically the largest source of acrolein exposure (Stevens and Maier 2008). Consequently, the health impacts arising from the inhalation of acrolein are higher than those from other routes of exposure (Linhart et al. 1996). The amount of acrolein in cigarette smoke can vary from 18 – 98 μg/cigarette (Roemer et al. 2004). Carbohydrates, mainly sugar additives amount for 48 – 98 mg/g of cigarette (Wang and Watson 2012), are a major source of acrolein in cigarette smoke (Roemer et al. 2012; Stevens and Maier 2008). Acrolein is a toxic respiratory irritant that has been estimated to account for 97% of total non-cancer respiratory hazard of mainstream cigarettes smoke (Fowles and Dybing 2003). The United States Environmental Protection Agency (US EPA) has determined that environmental exposure to acrolein is the leading cause for most non-cancer respiratory health effects (EPA 2002) in the national level. Furthermore, a recent study on acrolein exposure and asthma prevalence of the U.S. adult population (year 2000-2009) estimated that chronic exposure to outdoor acrolein at concentrations of 0.05 - 0.46 $\mu g/m^3$ was associated with an 8% increase in the prevalence-odds of having at least one asthma attack in the previous year (Decastro 2014).

Acrolein can also be formed endogenously as a product of polyamine metabolism and oxidative stress (Esterbauer et al. 1991; Igarashi and Kashiwagi 2011; Leung et al. 2011; Shi et al. 2011; Zhu et al. 2011). There is increasing evidence that acrolein formed endogenously is causally

involved in physiological effects such as inflammation, atherosclerosis, cardiovascular and neurodegenerative diseases, and cancer (Gueraud et al. 2010; Lopachin et al. 2008; Tang et al. 2011; Wu et al. 2011). Levels of protein-conjugated acrolein in plasma and in the brain have been reported to be higher among subjects with mild cognitive impairment and Alzheimer's disease than among age-matched normal control subjects (Bradley et al. 2010; Waragai et al. 2012). A recent study has observed an inverse correlation between brain infarction and urinary *N*-Acetyl-S-(3-hydroxypropyl)-L-cysteine (3HPMA), supporting the idea that glutathione plays an important role in detoxification of acrolein in cellular levels (Tomitori et al. 2012; Yoshida et al. 2012). Feng et al. suggest that acrolein is a major etiological agent for cigarette smoke related lung cancer and that it contributes to lung carcinogenesis by DNA damage and inhibition of DNA repair (Feng et al. 2006).

During 2012-2013 an estimated 50 million people, or 19.2% of all adults (aged 18 years and older) in the United States, used any combustible tobacco product every day (72.1% of those used ≥ 1 combustible tobacco products daily) or some days (CDC 2008). Cigarette smoking is the leading cause of preventable death in the United States, accounting for approximately 480,000 deaths, or 1 of every 5 deaths each year (DHHS 2014). Additionally, second- and third-hand smoke affects many U.S. residents, including a disproportionate number of children and infants (DHHS 2006, 2014; Pirkle et al. 2006; Protano et al. 2012). The tobacco product regulation group (TobReg) of the World Health Organization has identified acrolein as a major contributor to tobacco smoke toxicity (Burns et al. 2008).

N-Acetyl-S-(3-hydroxypropyl)-L-cysteine (3HPMA) and *N*-acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA) are specific urinary biomarkers of acrolein exposure (Figure 1). The main pathway of elimination of acrolein from human body is conjugation with glutathione (GSH) in

the liver followed by enzymatic cleavage and *N*-acetylation to form S-(3-oxopropyl)-*N*-acetyl-cysteine (OPMA) in the kidney (Kaye 1973). Reduction of the aldehyde group of OPMA forms 3HPMA (*N*-acetyl-S-[3-hydroxypropyl]-L-cysteine), the major urinary metabolite of acrolein exposure, and the oxidation of the aldehyde group of OPMA forms CEMA (*N*-acetyl-S-[2-carboxyethyl]-L-cysteine) as a minor metabolite.

Although a number of small scale studies have been carried out to evaluate acrolein exposure (Carmella et al. 2007; Carmella et al. 2009; Eckert et al. 2011; Schettgen et al. 2008), there have been no large scale biomonitoring studies reported for the general population. Therefore, as part of the National Health and Nutrition Examination Survey 2005-2006, urine samples were assayed a) to evaluate 3HPMA and CEMA in urine as biomarkers of exposure to acrolein for the U.S. population by age, sex, race, and smoking status; and b) to assess tobacco smoke as a predictor of acrolein exposure.

Methods

Study Design. NHANES is a population-based survey designed to assess the health and nutrition status for a representative sample of the civilian, non-institutionalized population of the United States. NHANES is conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). Spot urine samples were collected from a one-half subsample of NHANES 2005-2006 study participants 12 years and older. The study protocol was reviewed and approved by the CDC institutional review board; additionally, informed written consent was obtained from all subjects before they took part in the study. The characteristics of the population for which the acrolein exposure was measured are given in Table 1.

Analytical method. Residual *s*pot urine samples of NHANES 2005-2006 stored at -70 °C for 4-5 years were analyzed for 3HPMA and CEMA for the current study. An aliquot of 100 µl of each sample was assayed for 28 volatile organic metabolites using an ultra high performance liquid chromatography system (Waters Inc., Milford, MA) coupled with electro spray tandem mass spectrometry (Sciex API 4000 Triple Quad, Applied Biosystems, Foster City, CA) method (UPLC/ESI-MSMS) (Alwis et al. 2012). Urine samples were assayed at 1:10 dilution (100 µL urine + 50 µL working mixed internal standard + 850 µL 15 mM ammonium acetate). A mixture of 28 internal standards labeled with stable isotopes was used to quantify the levels. Chromatographic separation was achieved using an Acquity UPLC® HSS T3 1.8 µm x 2.1 mm x 150 mm (Waters Inc, Milford, MA) column with 15 mM ammonium acetate pH 6.8 (Solvent A) and acetonitrile (Solvent B) as mobile phases. The eluent from the column was ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope labeled internal standard) with known standard concentrations yielded individual analyte concentrations for unknowns. The Analyst software (version 1.5.1, Applied Biosystems, Foster City, CA) was used to operate both the UPLC and the API4000. The mass spectrometer was operated under scheduled multiple reaction monitoring (SMRM) mode for negative ions, the ion source temperature was kept at 650°C, and the electro spray ion voltage at -4000 V. The mass parameters were optimized for each analyte. For 3HPMA, m/z 220/91 and m/z 220/89 were monitored as quantitation and confirmation ion transitions respectively. For CEMA, m/z 234/162 (quantitation ion transition) and m/z 234/105 (confirmation ion transition) were monitored. We monitored m/z 226/97 for $^{2}H_{6}$_3HPMA and m/z 237/162 for $^{13}C_{3}$_CEMA. The limits of detection (LOD) for 3HPMA and CEMA were 13 and 8 ng/mL respectively.

Statistics. The NCHS has conducted NHANES since the early 1960s in order to assess the health and nutritional status of the United States through the collection of serial cross sectional data from a complex, multistage probability sample representative of the civilian, non-institutionalized population of the United States (CDC 2005-2006). 3HPMA and CEMA were measured in a single spot urine sample obtained from participants ≥ 12 years and older during physical examinations carried out in a mobile examination center. Reported results met the accuracy and precision specification of the quality control/quality assurance program of the CDC National Center for Environmental Health, Division of Laboratory Sciences (Caudill et al. 2008). Measurements below the limit-of-detection were substituted with the quotient of the limit-of-detection divided by the square-root of two.

Acrolein biomarkers, 3HPMA and CEMA were measured in urine collected from 3,545 NHANES participants. Acrolein is present in tobacco smoke, so NHANES participants who recently smoked tobacco were distinguished from non-tobacco users when serum cotinine (a nicotine-specific biomarker, measured in a serum sample obtained at the same time as the spot urine sample) exceeded 10 ng/ml (Pirkle et al. 1996). There were 271 participants excluded from the analytical data set because they were without serum cotinine data. In addition, to focus analysis on combustible tobacco product smokers (named as “tobacco smokers” in the rest of this document), participants exceeding 10 ng/ml serum cotinine were included in the analytical dataset if they also responded "yes" to NHANES smoking questionnaire question SMQ680 (tobacco or nicotine use within 5 days prior to NHANES physical examination), also responded "yes" to at least one of SMQ690A - SMQ690C (cigarettes, pipes, cigars), and responded "no" to all SMQ690D - SMQ690F (smokeless tobacco and nicotine delivery products). Non-tobacco users had serum cotinine ≤ 10 ng/ml and answered “no” to SMQ680. Another 172 participants

were excluded for not having answered SMQ680, and another 206 participants were excluded because data were missing for other variables using in the regression models, leaving 2866 for statistical analysis.

Because NHANES participants are recruited through a multistage sampling design, it is necessary to account for this complex design in order to properly estimate variances and to produce unbiased, nationally representative statistics. Robust estimation may be accomplished through a generalized estimation equations approach incorporating Taylor series linearization and applying survey sample weights to each survey participant. We used this estimation approach as it was implemented in the DESCRIPT and REGRESS subroutines of SUDAAN version 11.0.0 (Research Triangle Institute 2012) called from the SAS version 9.3 statistical software application. Sample-weighted linear regression models stratified by tobacco use status (tobacco smokers vs. non-tobacco users) were fit to NHANES data from the 2005 – 2006 survey cycles (CDC 2005-2006) where the dependent variable is the urinary concentration of 3HPMA or CEMA (μg/L). Because the distribution of urinary measurements was highly right skewed, which would have adversely affected hypothesis testing, urinary concentration data were transformed with the natural log for the purpose of evaluating the statistical significance of regression slopes. The *p*-values for slopes from the ln urinary concentration regression models are reported. To facilitate interpretability, however, we report slopes and their 95% confidence intervals estimated from identical regression models of untransformed urinary concentration data. Statistical significance was set to $\alpha \leq 0.05$, and marginal significance was set to $0.05 < \alpha \leq 0.15$.

Additional self-reported predictors included in the regression models were sex, age, race / ethnicity. Age (yr) was divided into the following ranges: 12 – 19, 20 – 39, 40 – 59, and ≥ 60.

Serum cotinine was used as a continuous variable to evaluate the association between urinary concentration of 3HPMA and CEMA and tobacco smoke exposure in the regression model for both tobacco smokers and non-tobacco users. Among non-tobacco users tobacco smoke exposure is primarily attributable to secondhand smoke (SHS), which is associated with serum cotinine. In order to directly associate urinary biomarker concentrations with the frequency of cigarette smoking, we ran the same regression model for those reporting exclusive use of cigarettes, but replaced serum cotinine with self-reported average number of cigarettes smoked per day (CPD) over the five days preceding the MEC exam, classified in ranges of 1 - 10 CPD (0.5 pack), 11 - 20 (1 pack), 21 - 30 (1.5 packs) and >30 (>1.5 packs), where the reference category comprised self-reported non-tobacco users with serum cotinine $\leq$ LOD (0.015 ng/mL).

3HPMA and CEMA respectively are major and minor metabolites of acrolein exposure. Both are formed from the same intermediate S-(3-oxopropyl)-*N*-acetyl-cysteine (OPMA). 3HPMA is the major metabolite. Smoking can elevate the urinary biomarker levels. There can also be variation of enzyme activity metabolizing acrolein to 3HPMA and CEMA among races. To investigate race differences in acrolein metabolism we calculated sample weighted CEMA/3HPMA molar ratio by smoking status and race/ethnicity for the NHANES 2005-2006 population.

Since urinary biomarker concentrations can be influenced by urine dilution, which can vary markedly throughout the day within each individual, statistical inference can be confounded (Barr et al. 2005). Urine dilution can be accounted for by scaling urinary analyte concentration to the urinary concentration of creatinine, a compound excreted endogenously at a fairly constant rate and therefore resistant to urine dilution. Summary statistics for urine 3HPMA and CEMA according to demographic characteristics and tobacco use are reported as the urinary concentration ratio of 3HPMA and CEMA to creatinine (μg/g creatinine), whereas regression

models used to estimate associations between urine 3HPMA and CEMA concentrations included urinary creatinine (g/mL) as a covariate to account for urine dilution.

Results

3HPMA and CEMA were detected in 99 and 98%, respectively, of the samples assayed. Urinary 3HPMA and CEMA levels were higher among tobacco smokers than non-tobacco users ($p < 0.0001$) for the NHANES 2005 - 2006 population (Table 2, for geometric means and percentiles, see Supplemental Material, Tables S1 and S2). Our multiple regression model of urinary 3HPMA among tobacco smokers (Table 3), adjusted for sex, age, and race/ethnicity, showed that serum cotinine was positively associated with 3HPMA levels in urine ($p < 0.0001$). 3HPMA was significantly lower among 12-19 and 20-39, year-olds compared to 40-59 year-olds; and Mexican Americans, non-Hispanic Blacks and Other Hispanics/Other Multi-race had significantly lower 3HPMA than non-Hispanic whites. A multiple regression model for urinary CEMA among tobacco smokers (Table 3) had similar results: serum cotinine was positively associated with CEMA levels in urine ($p < 0.0001$); CEMA was significantly lower among 12-19 and 20-39 year-olds compared to 40-59 year-olds; and Mexican Americans had significantly lower CEMA than non-Hispanic whites. In contrast with 3HPMA, there were no significant differences in CEMA levels among non-Hispanic Blacks and other race/ethnicity compared with non-Hispanic Whites. Among non-tobacco users, our regression model data showed that serum cotinine was a significant predictor of CEMA ($p = 0.007$) but not 3HPMA ($p = 0.8$) (see Supplemental Material, Table S3).

The associations between urinary biomarkers and cigarettes smoked per day (CPD) for exclusive cigarette smokers were also examined for the same population. Figure 2 displays the least-square mean of the natural log of urinary 3HPMA and CEMA concentration for each CPD category,

adjusted for sex, age, race/ethnicity, and urinary creatinine. Both 3HPMA and CEMA levels increased with increasing number of cigarette smoked per day among exclusive cigarette smokers. The multiple regression models of urinary 3HPMA and CEMA among exclusive cigarette smokers, adjusted for sex, age, and race/ethnicity (see Supplemental Material, Table S4), also showed that both urinary 3HPMA and CEMA increased in a dose-dependent manner across participants who smoked 1- 10, 11-20, 21-30, and > 30 cigarettes per day compared to those with serum cotinine ≤ LOD of 0.015 ng/mL. Among age groups, 3HPMA was significantly lower for 12-19, 20-39, and ≥ 60 year-olds compared to 40-59 year-olds. CEMA was significantly lower among 12-19 and 20-39 year-olds compared to 40-59 year-olds. According to NHANES 2005-2006 demographic information, among smokers (serum cotinine > 10 ng/mL), 43% of the participants were in 1-10 CPD (0.5 pack), 35% in 11-20 CPD (1 pack), 9% in 21-30 CPD (1.5 packs), and 6% in > 30 CPD (> 1.5 packs) category.

The CEMA/3HPMA molar ratio was significantly higher for non-tobacco users compared to tobacco smokers ($p < 0.001$) and varied among race/ethnic groups within smoking status categories (Table 4). Non-Hispanic Blacks had significantly higher CEMA/3HPMA ratios for both tobacco smokers and non-tobacco users compared to non-Hispanic whites. Among non-tobacco users, Mexican-Americans and other Hispanic or other/multi-race participants had significantly lower CEMA/3HPMA ratios than non-Hispanic Whites ($p = 0.003$ and 0.03, respectively), and among tobacco smokers other Hispanic or other/multi-race participants had significantly higher CEMA/3HPMA ratios than non-Hispanic Whites ($p = 0.03$).

We used serum cotinine to categorize tobacco smokers from non-tobacco users for NHANES 2005-2006 samples. In unweighted analysis, significant correlations ($p < 0.0001$) were found between serum cotinine and urinary acrolein biomarkers among tobacco smokers ($r = 0.52$ for

3HPMA and serum cotinine; $r = 0.45$ CEMA and serum cotinine). The unweighted correlation between 3HPMA and CEMA was strong among tobacco smokers ($r = 0.77$, $p < 0.0001$) compared to non-tobacco users ($r = 0.46$, $p < 0.0001$). The percent distribution of urinary 3HPMA and CEMA among tobacco smokers and non-tobacco users for the U.S. population is shown in Figure 3.

Discussion

Acrolein is a reactive aldehyde which is also a toxic respiratory irritant, cardiotoxicant, and carcinogen. Among exogenous sources, tobacco smoke is a major source of exposure to acrolein (Stevens and Maier 2008; Stevens and Maier 2011). The human body detoxifies acrolein by conjugating it with glutathione, producing 3HPMA and CEMA as major and minor byproducts, respectively (Stevens and Maier 2011). Consistent with the published literature, we observed that the median 3HPMA level (1089 μg/g creatinine) measured was 5 times greater than that of the median level for CEMA (203 μg/g creatinine) among tobacco smokers. Significant associations found between urinary 3HPMA and CEMA with serum cotinine among tobacco users and the dose-dependent relationships between biomarkers with CPD among exclusive cigarette smokers show that 3HPMA and CEMA are effective urinary biomarkers of tobacco smoke related acrolein exposure. The biomarker levels were significantly higher among tobacco smokers compared to non-tobacco users.

3HPMA and CEMA are formed from the intermediate OPMA (S-(3-oxopropyl)-*N*-acetyl-cysteine). The reduction of the aldehyde group of OPMA by the aldo-keto reductase enzyme forms 3HPMA, whereas CEMA is formed by the oxidation of the aldehyde group of OPMA by the aldehyde dehydrogenase enzyme. Because both are formed from the same intermediate compound, it is possible to expect a significant difference in CEMA/3HPMA molar ratio among

tobacco smokers and non-tobacco users. 3HPMA is the major urinary metabolite of acrolein exposure (Stevens and Maier 2008) and the levels were 5 times greater than urinary CEMA levels among tobacco smokers for this study population. Among non-tobacco users 3HPMA levels were approximately 3 times greater than CEMA levels. Therefore the ratio was significantly higher ($p < 0.0001$) among non-tobacco users (0.32) compared to tobacco smokers (0.18). We also found significantly higher CEMA/3HPMA ratio for non-Hispanic Blacks compared to non-Hispanic Whites. Currently there is no information about CEMA/3HPMA ratio or the variation of enzyme activity metabolizing acroelin to mercapturic acids among races in the literature. Our findings may help future research to understand the phase II detoxification of acrolein by enzymes, the inhibitors and inducers of enzyme activity and the variation of enzyme activity metabolizing acrolein among races.

The measured 3HPMA levels in our study were about four times higher in tobacco smokers than non-tobacco users, consistent with all but one previous study (Schettgen et al. 2008) (Table 5). Our study reported median urinary 3HPMA levels of 219 and 1089 μg/g creatinine for non-tobacco users and tobacco smokers, respectively. A European study (Eckert et al. 2011) has also reported similar median values: 146 and 884 μg/g creatinine, respectively, for non-smokers and smokers. Similar mean 3HPMA levels were observed for tobacco smokers and non-tobacco users by two large scale studies (the current study and Roethig et al., 2009) investigating volatile organic compound exposure among the US population (Table 5).

Conclusions

The NHANES 2005-2006 3HPMA and CEMA data provide the first reference levels of acrolein exposure for the US population against which subsequent NHANES and other large scale epidemiologic studies may be compared. These levels of urinary biomarkers 3HPMA and

CEMA characterize acrolein exposure in the U.S. population. Acrolein, a combustion product in tobacco smoke has been estimated to account for 97% of total non-cancer respiratory hazard of mainstream cigarette smoke (Fowles and Dybing 2003). Although the prevalence of cigarette smoking among the U.S. adults in 2012-2013 (18%) was significantly lower compared to prevalence observed in 2009-2010 (19.5%), cigarettes and other combustible products remained the most prevalent forms of adult tobacco use in the United States (CDC 2008). Future efforts will explore acrolein exposure from other sources (e.g. acrolein in indoor and outdoor air), investigate the association of acrolein exposure and health effects, and evaluate the efficacy of strategies for reducing the levels from major sources such as tobacco smoke.

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Table 1. Sample-weighted demographic proportions of the NHANES 2005-2006 participants (sample sizes are unweighted).

Variable	**Non-tobacco users (NTU)[a]**		**Tobacco smokers (TS)[b]**	
	n[c]	**% (SE)[d]**	**n**	**% (SE)**
Age (yr)				
12 - 19	752	14.9 (0.91)	104	7.16 (0.93)
20 - 39	563	28.9 (1.52)	205	44.4 (3.48)
40 - 59	459	33.9 (1.56)	172	36.5 (3.32)
≥ 60	522	22.2 (2.22)	89	12.0 (1.70)
Race/ethnicity				
Mexican-American	628	9.41 (1.25)	78	4.88 (1.20)
Non-Hispanic Black	558	10.3 (2.01)	178	13.5 (2.24)
Non-Hispanic White	942	71.1 (2.92)	275	73.8 (3.35)
Other Hispanic or Other/multi race	168	9.18 (1.51)	39	7.83 (1.85)
Sex				
Female	1288	54.9 (0.97)	248	45.7 (2.00)
Male	1008	45.1 (0.97)	322	54.3 (2.00)

[a]Non-tobacco users (NTU): defined for this study as non-smokers with serum cotinine ≤ 10 ng/mL.

[b]Tobacco smokers (TS): defined for this study as smokers with serum cotinine > 10 ng/mL, using cigarettes, cigars, or pipes during last 5 days prior to physical examination.

[c]Sample size.

[d]Standard error.

Table 2. Sample-weighted median (25^{th}, 75^{th} percentile) urinary 3HPMA and CEMA concentrations (creatinine adjusted) by age, sex and race/ethnicity categorized by smoking status among NHANES 2005-2006 participants $\geq$ 12 years-old (sample sizes are unweighted).

Variable	Sample size (n)		3HPMA (25^{th}, 75^{th} percentile) (µg/g creatinine)		CEMA (25^{th}, 75^{th} percentile) (µg/g creatinine)	
	NTU	TS	NTU	TS	NTU	TS
All	2,467	601	219 (140, 353)	1089 (469, 2012)	78.8 (51.8, 121)	203 (111, 338)
Age (yr)						
12 - 19	811	114	192 (130, 285)	477 (333, 755)	65.5 (44.5, 103)	122 (83.3, 202)
20 - 39	618	208	216 (136, 348)	836 (430, 1641)	71.8 (49.0, 104)	157 (97.1, 253)
40 - 59 [Ref]	493	177	239 (146, 400)	1602 (691, 2714)	82.2 (50.8, 130)	246 (142, 407)
≥ 60	545	102	215 (140, 332)	1375 (702, 2345)	94.2 (62.8, 146)	309 (170, 415)
Race/ethnicity						
Mexican American	685	82	243 (154, 410)	445 (254, 843)	78.3 (52.4, 121)	102 (69.6, 171)
Non-Hispanic Black	613	191	199 (128, 299)	741 (409, 1342)	81.9 (53.9, 130)	191 (111, 297)
Non-Hispanic White [Ref]	984	287	216 (137, 345)	1248 (519, 2255)	78.4 (51.2, 119)	212 (119, 346)
Other Hispanic or Other/multi race	185	41	261 (175, 400)	1094 (370, 1687)	80.8 (53.7, 122)	178 (104, 347)
Sex						
Female [Ref]	1,379	252	211 (132, 369)	1269 (579, 2248)	80.5 (50.7, 127)	233 (116, 378)
Male	1,088	349	233 (148, 344)	932 (432, 1796)	77.8 (53.1, 116)	178 (108, 298)

Table 3. Sample-weighted multiple regression slopes for urinary 3HPMA and CEMA concentrations among NHANES 2005-2006 tobacco smokers.

Variable	**3HPMA Coefficient (95% CI)[a]**	***P*[b]**	**CEMA Coefficient (95% CI)**	***P*[b]**
Intercept	-90.8 (-692, 510)	<.0001	-12.4 (-60.5, 35.7)	<.0001
Serum cotinine (ng/mL)	5.05 (3.35, 6.74)	<.0001	0.63 (0.41, 0.84)	<.0001
Creatinine (g/mL)	1117490 (757159, 1477821)	<.0001	200849 (150890, 250808)	<.0001
Age (yr)				
12 - 19	-947 (-1358, -536)	0.002	-117 (-205, -28.7)	0.01
20 - 39	-658 (-965, -350)	0.006	-112 (-179, -44.6)	0.001
40 - 59 [Ref]	Ref.	.	Ref.	.
≥ 60	-318 (-773, 138)	0.23	26.1 (-31.4, 83.7)	0.88
Sex				
Female [Ref]	Ref.	.	Ref.	.
Male	-189 (-391, 13.3)	0.69	-25.5 (-74.5, 23.5)	0.79
Race/ethnicity				
Mexican American	-556 (-991, -121)	0.003	-74.1 (-125, -23.3)	0.0006
Non-Hispanic Black	-902 (-1188, -615)	<.0001	-20.7 (-98.5, 57.1)	0.67
Non-Hispanic White [Ref]	Ref.	.	Ref.	.
Other Hispanic or other/multi race	-751 (-1316, -186)	0.02	-70.1 (-169, 29.1)	0.29

[a](95% CI)= 95% confidence intervals. [b]*p* value was estimated from identical models where the dependent variable was natural log-transformed.

Table 4. Sample-weighted molar ratios of urinary acrolein metabolites (CEMA/3HPMA) by smoking status and race/ethnicity.

Variable	GM (95% CI)[a]	Median (25^{th}, 75^{th} percentile)	*p*
Race/ethnicity			
Tobacco smokers (TS)			
Mexican American	0.20 (0.14, 0.26)	0.21 (0.14, 0.33)	0.18
Non-Hispanic Black	0.24 (0.22, 0.26)	0.23 (0.16, 0.36)	<.0001
Non-Hispanic White	0.17 (0.15, 0.18)	0.17 (0.12, 0.25)	Ref.
Other Hispanic or other multi race	0.21 (0.16, 0.26)	0.20 (0.13, 0.31)	0.03
Non-tobacco users (NTU)			
Mexican American	0.27 (0.24, 0.30)	0.28 (0.18, 0.46)	0.003
Non-Hispanic Black	0.38 (0.36, 0.41)	0.39 (0.25, 0.59)	0.004
Non-Hispanic White	0.33 (0.31, 0.35)	0.34 (0.21, 0.52)	Ref.
Other Hispanic or Other/multi race	0.28 (0.25, 0.32)	0.28 (0.18, 0.46)	0.03
Serum cotinine			
≤ 10 ng/mL	0.32 (0.31, 0.34)	0.33 (0.21, 0.52)	<.0001
> 10 ng/mL	0.18 (0.17, 0.20)	0.18 (0.13, 0.29)	Ref.

[a]GM (95% CI) = Geometric mean (95% confidence intervals).

Table 5. Comparison of urinary biomarker levels among smokers and non-smokers.

Source	No. of subjects		3HPMA		CEMA	
	NTU	TS	NTU	TS	NTU	TS
Mean µg/g creatinine						
Current study	2467	600	309	1510	99.4	248
Roethig et al. 2009[a]	1077	3585	327	1450		
Scherer et al. 2007[b]	100	194	241	926		
Mascher et al. 2001[c]	41	27	580	2006		
Median (µg/g creatinine)						
Current study	2467	600	219	1089	78.8	203
Eckert et al. 2011[d]	54	40	146	884		
Schettgen et al. 2008[e]	14	14	113	1630		
Carmella et al. 2007[f]	21	35	151	643		

[a]Smokers: defined as regular consumption of a minimum of one manufactured cigarette per day over the last 12 months. Non-smokers: defined as no use of tobacco or nicotine containing products during the five years prior to the study (Roethig et al. 2009).

[b]Smokers: self-reported daily cigarette users; cotinine and trans-3-hydroxy cotinine were analyzed in saliva of both smokers and non-smokers; nicotine equivalents and NNAL were analyzed in smoker urine samples as part of the study (Scherer et al. 2007).

[c]Self-reported (Mascher et al. 2001).

[d]Smoking status was verified by urine cotinine analysis in addition to self-reported smoking behavior: Smoker urinary cotinine levels ranged from 0.7-136 µg/L and non-smoker levels ranged from 52.4 -3752 µg/L (Eckert et al. 2011).

[e]Smoking status was verified by urine cotinine analysis in addition to self-reported smoking behavior: Smoker urinary cotinine levels ranged from 713-3073 µg/L and non-smoker level ranged < 1-22 µg/L (Schettgen et al. 2008).

[f]Self-reported (Carmella et al. 2007).

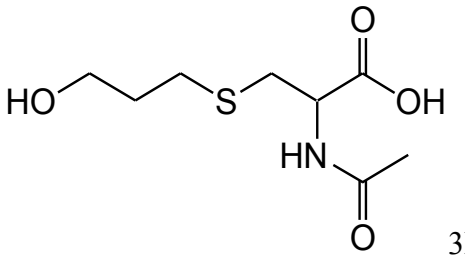
Figure Legends

Figure 1. *N*-Acetyl-S-(3-hydroxypropyl)-L-cysteine (3HPMA) and *N*-Acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA) are urinary biomarkers of acrolein exposure.

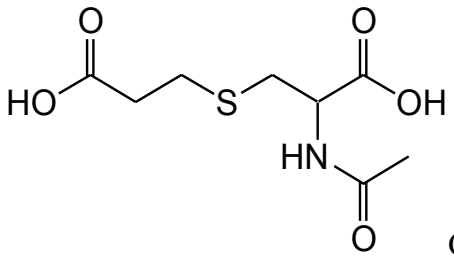
Figure 2. Least-square mean [95% confidence intervals] for each cigarette smoked per day (CPD) category of urinary 3HPMA (a) and CEMA (b) concentrations among exclusive cigarette smokers with serum cotinine >10 ng/ml, adjusted for sex, age, race/ethnicity, and urinary creatinine.

Figure 3. The percent distribution of urinary 3HPMA and CEMA (μg/g creatinine) among tobacco smokers and non-tobacco users: NHANES 2005-2006 [percentages not sample weighted].

Figure 1.



3HPMA



CEMA

Figure 2.

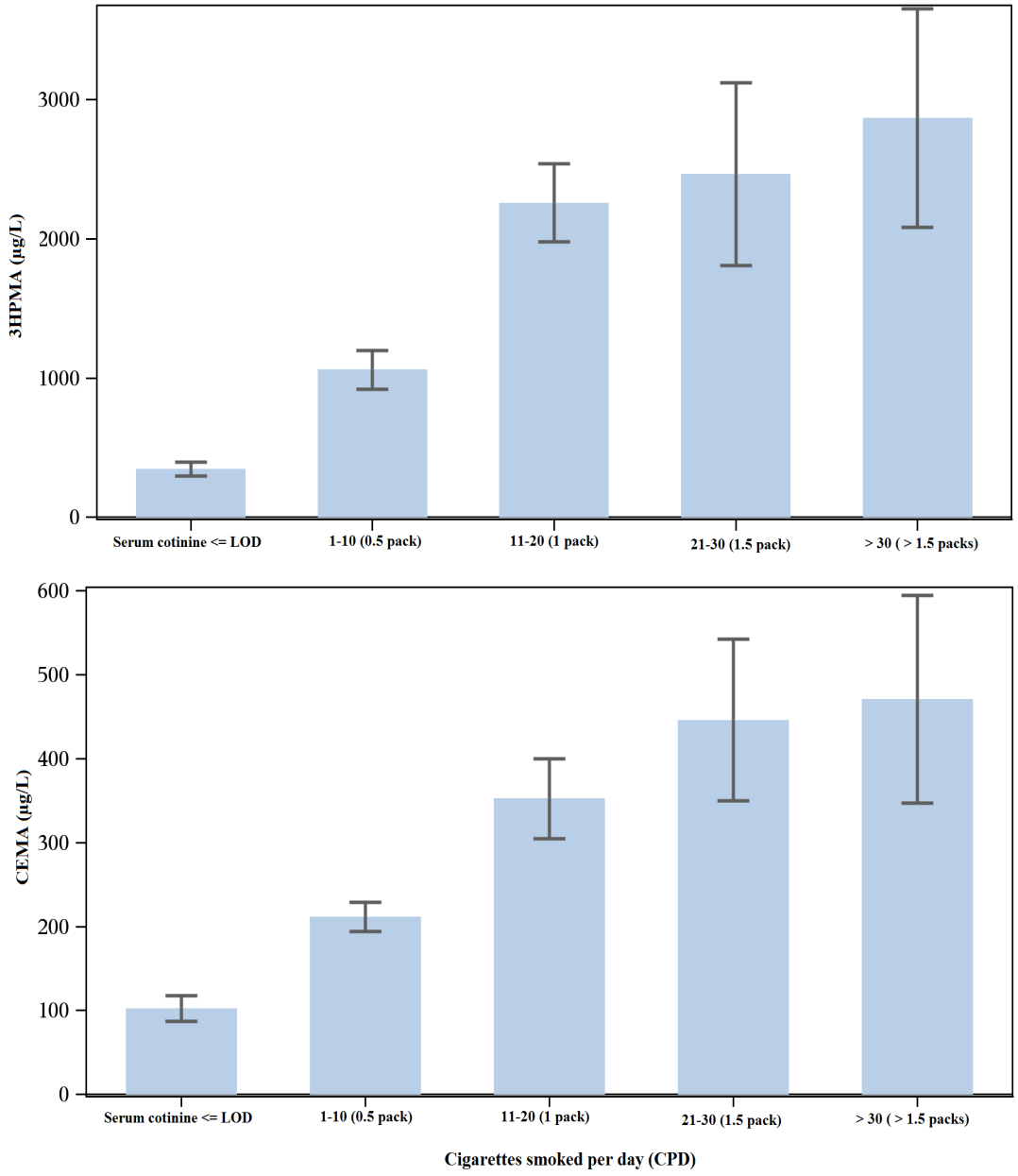
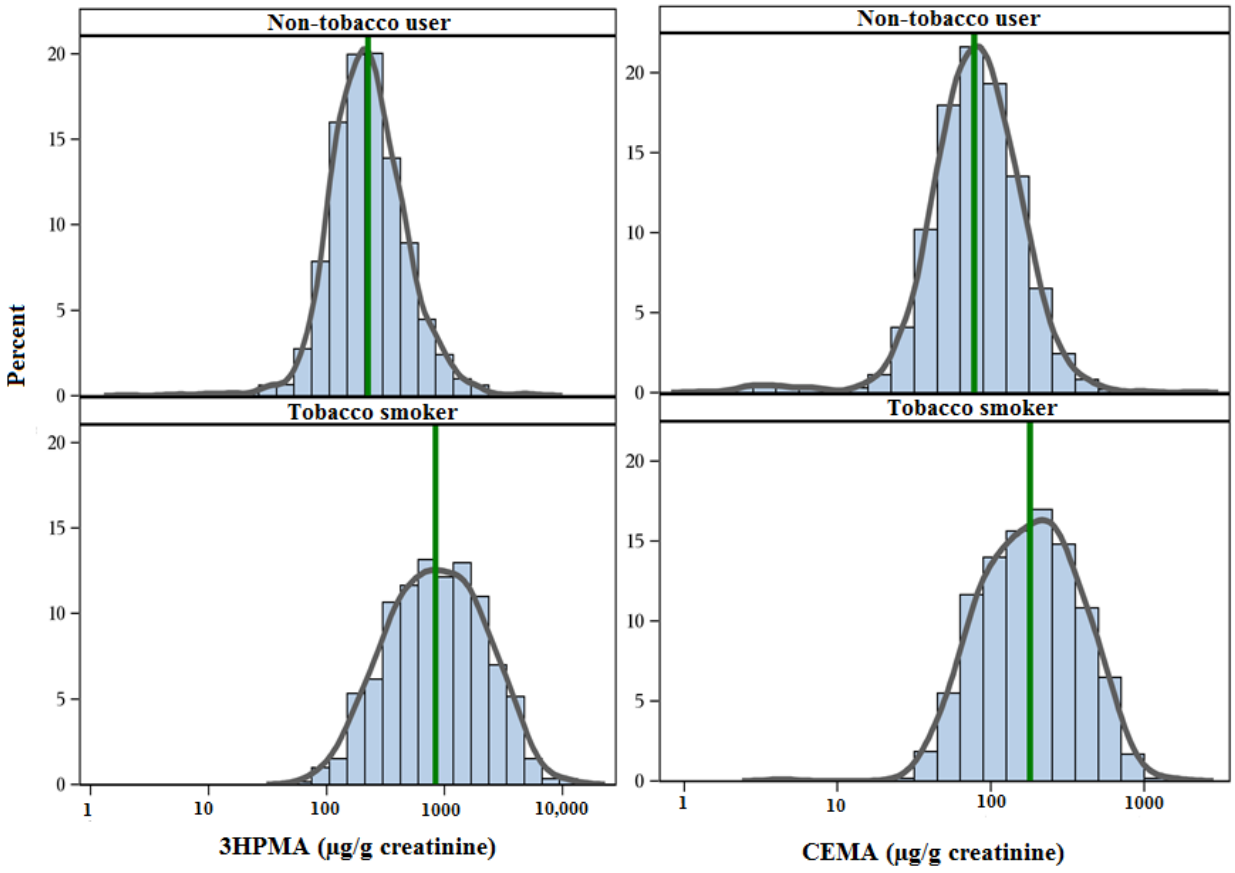

3HPMA (µg/L)
3000
2000
1000
0
Serum cotinine <= LOD
1-10 (0.5 pack)
11-20 (1 pack)
21-30 (1.5 pack)
> 30 (> 1.5 packs)
(a)
CEMA (µg/L)
600
500
400
300
200
100
0
Serum cotinine <= LOD
1-10 (0.5 pack)
11-20 (1 pack)
21-30 (1.5 pack)
> 30 (> 1.5 packs)
(b)
Cigarettes smoked per day (CPD)

Figure 3.


Non-tobacco user
Tobacco smoker
Percent
0
5
10
15
20
1
10
100
1000
10,000
3HPMA (µg/g creatinine)
CEMA (µg/g creatinine)